

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

## PKM2, STAT3 and HIF-1 $\alpha$ : the Warburg's vicious circle.

### This is the author's manuscript

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/126933> since

*Published version:*

DOI:10.1016/j.molcel.2012.01.001

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

## **PKM2, STAT3 and HIF-1 $\alpha$ : the Warburg's vicious circle**

Marco Demaria<sup>1</sup>, Valeria Poli<sup>2</sup>

*<sup>1</sup>Buck Institute for Research on Aging, Novato CA, USA; <sup>2</sup>Molecular Biotechnology Center and Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy;*

**KEYWORDS:** pyruvate kinase isoforms, PKM2, glycolysis, Warburg, cancer, cell proliferation, malignant transformation, growth factors, STAT3

**ABBREVIATIONS:** TCA, Tricarboxylic Acid Cycle; PKM, Pyruvate Kinase M-type; NADPH, nicotinamide adenine dinucleotide phosphate; IL-3, Interleukin-3; EGFR, Epidermal Growth Factor Receptor; CCND1, Cyclin D1 gene; HIF-1 $\alpha$ , Hypoxia-Inducible Factor-1 $\alpha$ ; MEK5, Mitogen-activated protein kinase kinase 5; STAT3, Signal Transducer and Activator of Transcription 3; PEP, Phosphoenolpyruvic acid; ADP, Adenosine Diphosphate; IL-6, Interleukin-6; NF $\kappa$ B, Nuclear Factor-KappaB

## **ABSTRACT**

The M2 isoform of pyruvate kinase, highly expressed in tumor cells, is known to engage a feed forward loop with the glycolysis master transcription factor HIF-1 $\alpha$ . Gao and co-authors recently showed that dimeric PKM2 localizes to the nucleus in highly proliferating cancer cells, where it regulates *in vivo* growth by acting as a protein kinase and directly activating STAT3. STAT3 is therefore a novel player of the PKM2/HIF-1 $\alpha$  feedback loop, since HIF-induced PKM2 activates STAT3 that in turn induces HIF-1 $\alpha$  expression. These findings have profound implications for understanding the complex connections between gene regulation, metabolism, survival and proliferation in cancer.

## TEXT

Pyruvate kinase (PK) catalyzes the penultimate step of glycolysis by transferring phosphate from phosphoenolpyruvate (PEP) to ADP to generate ATP and pyruvate, which can then be converted in either lactate or acetyl-CoA to enter the TCA cycle. Of the four known isoforms, PKM1 and PKM2 derive from an alternative splicing of the same *pkm2* gene.<sup>1</sup> PKM2 is highly expressed in embryonal cells and in tumors, and its prevalent expression in cancer cells has been linked to lower O<sub>2</sub> consumption and higher lactate secretion and glucose intake. The switch to the PKM2 isoform, observed in tumors of different origin,<sup>2, 3</sup> was shown to be essential for both the ‘Warburg’ effect and proliferation of cancer cells.<sup>4, 5</sup>

The main feature distinguishing PKM2 from the PKM1 isoform is the fact that its pyruvate kinase activity is subject to multiple layers of regulation. PKM2 can exist as a tetramer, which is enzymatically active, or as an inactive dimer. Interestingly, tumors show very high levels of dimeric PKM2, which in normal proliferating cells is in contrast mainly tetrameric.<sup>6</sup> Multiple signals such as growth factors, oncogenes or reactive oxygen species (ROS) were shown to destabilize the tetrameric form via a number of mechanisms including tyrosine phosphorylation, association with tyrosine phosphopeptides or oxidation.<sup>7-9</sup> In turn, decreased PKM2 activity correlates with increased proliferation. Although this can be partly explained by the diversion of glucose metabolites from catabolic to anabolic processes<sup>10</sup> and by the increased generation of anti-oxidant NADPH via the pentose phosphate pathway,<sup>9</sup> a consistent body of data points towards an independent role for PKM2 in the nucleus. Indeed, it was shown that both IL-3 and EGFR can induce PKM2 nuclear localization, correlating with cell proliferation.<sup>11, 12</sup> Nuclear PKM2 can associate with cSrc-phosphorylated beta-catenin, and bind to the *ccnd1* gene promoter activating cyclin D1 transcription.<sup>12</sup> Finally, PKM2 was recently proposed to be part of a “feed forward loop” enhancing the activity of

hypoxia-inducible factor (HIF)-1, a key transcriptional regulator of both aerobic and anaerobic glycolysis.<sup>13</sup> Indeed, HIF-1 can activate *pkm2* gene transcription, and PKM2 in turn interacts with the HIF-1 $\alpha$  subunit and promotes trans-activation of HIF-1 target genes, thus enhancing cellular responses to oxygen deprivation or oncogene activation.

To investigate the functional significance of nuclear PKM2, Gao et al.<sup>14</sup> have compared PKM2 nuclear localization in different cancer cell lines characterized by differential proliferative ability. Interestingly, they observed a strong correlation between PKM2 nuclear levels, cell proliferation and *mek5* gene transcription. Upon showing by ChIP analysis that PKM2 associates to the *mek5* promoter, they went on demonstrating that nuclear PKM2 interacts with the transcription factor STAT3 and is able to directly trigger its activation via phosphorylation on tyrosine 705. The authors were also able to demonstrate that *mek5* is a direct STAT3 transcriptional target, and that the levels of dimeric PKM2 directly correlate with STAT3-mediated *mek5* transcription, cell proliferation and *in vivo* growth of tumor cells. Interestingly, PKM2-mediated STAT3 tyrosine phosphorylation can be accomplished *in vitro* exclusively by dimeric PKM2, which uses PEP as a phosphate donor and likely interacts with its substrate via the ADP binding domain. Incidentally, the reaction will lead to pyruvate production via a yet alternative pathway.

STAT3 is a point of convergence for many oncogenic signals, and its aberrant constitutive activity is crucial for the survival, proliferation and metastatic activity of tumors of different origin.<sup>15</sup> Moreover, STAT3 constitutive activity plays a key role in inflammation-induced cancer, by instating a feed forward loop entailing continuous production of the pro-inflammatory cytokine IL-6 and constitutive NF- $\kappa$ B activation.<sup>16</sup> We have recently shown that constitutively active STAT3 induces chronically enhanced *hif-1 $\alpha$*  transcription that results in increased HIF-1 $\alpha$  protein levels and triggers a metabolic switch towards aerobic glycolysis similar to the Warburg effect.<sup>17, 18</sup>

STAT3 activation appears thus to participate in the recently proposed PKM2/HIF-1 $\alpha$  positive feedback loop,<sup>13</sup> where oxygen deprivation or oncogenes lead to increased HIF-1 $\alpha$  levels, HIF-1 induces *pkm2* transcription, PKM2 enhances HIF-1 trans-activating power and activates STAT3, and finally activated STAT3 enhances HIF-1 $\alpha$  expression (Figure 1). On the other hand, we have recently shown that constitutively active STAT3 can act as a first hit during the process of malignant transformation.<sup>19</sup> Aberrantly continuous STAT3 activation like that found in chronic inflammation might therefore sensitize cells to tumor transformation by initiating the above described positive feedback loop, leading to enhanced HIF-1 $\alpha$  and PKM2 expression/activity. This would in turn support aerobic glycolysis, anabolic cell metabolism, cell survival and proliferation. Moreover, PKM2-mediated STAT3 phosphorylation may participate in the chronic STAT3 activation observed in highly glycolytic tumors of different origins. Although these findings shed some light on the correlation between the tetrameric:dimeric PKM2 ratio, its nuclear activities and enhanced cell proliferation, several questions still remain unanswered. For example, why does PKM2 preferentially localize in the nucleus in more proliferating cells? Is this a special feature of cancer cells? What is the mechanism that drives dimeric PKM2 to the nucleus? An intriguing possibility, adding one more layer of cross-talk, would be that the transcription factors/co-factors known to interact with PKM2, i.e. beta-catenin, HIF-1 $\alpha$  or STAT3, may mediate its transport to the nucleus. Another interesting implication of the findings by Gao et al. is the idea that STAT3 phosphorylation may take place in the nucleus, opposed to its canonical cytoplasmic activation pathway. This correlates with datasets showing that unphosphorylated STAT3 is continuously shuttling between the cytoplasm and the nucleus generating a reservoir of nuclear unphosphorylated STAT3 available for activation.<sup>20</sup> Additionally, the symultaneous detection of both PKM2 and STAT3 on the *mek5* promoter suggests that

nuclear phosphorylation of STAT3 by PKM2 might even occur on the DNA, similar to what has been recently shown for STAT3 serine phosphorylation.<sup>21</sup>

Small molecules enhancing PKM2 tetrameric activity have already been proposed as new therapeutic tools to compromise both the anabolic and the anti-oxidant functions of this protein.<sup>9</sup> The same compounds may also lead to the inhibition of PKM2-mediated STAT3 activation, potentially representing a useful therapeutic tool to help hitting the uncontrolled growth of those highly glycolytic tumors that display both STAT3 activation and dimeric PKM2.

## ACKNOWLEDGMENTS

Work in V. Poli's group is supported by the Italian Cancer Research Association (AIRC IG 9272), the Italian Ministry for University and Research (FIRB and MIUR), and the Truus and Gerrit van Riemsdijk Foundation, Vaduz, Liechtenstein.

## FIGURE LEGEND

**STAT3/PKM2/HIF-1 $\alpha$  feed forward loop.** Oxygen deprivation, growth factors or oncogenes lead to an increase in HIF-1 $\alpha$  levels, HIF-1 induces *pkm2* transcription, PKM2 both enhances HIF-1 trans-activating power and activates STAT3, and activated STAT3 induces HIF-1 $\alpha$  expression. On the other hand, cytokines, growth factors or oncogenes activate STAT3 that can induce *hif-1 $\alpha$*  transcription and start the positive feedback loop between STAT3, PKM2 and HIF-1 $\alpha$ . However initiated, this leads to increased levels of proteins involved in glycolysis, enhancing the anaerobic-like metabolism known as Warburg effect.

## REFERENCES

1. Noguchi T, Inoue H, Tanaka T. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J Biol Chem* 1986; 261:13807-12.
2. Brinck U, Eigenbrodt E, Oehmke M, Mazurek S, Fischer G. L- and M2-pyruvate kinase expression in renal cell carcinomas and their metastases. *Virchows Arch* 1994; 424:177-85.
3. Steinberg P, Klingelhoffer A, Schafer A, Wust G, Weisse G, Oesch F, et al. Expression of pyruvate kinase M2 in preneoplastic hepatic foci of N-nitrosomorpholine-treated rats. *Virchows Arch* 1999; 434:213-20.
4. Warburg O. On respiratory impairment in cancer cells. *Science* 1956; 124:269-70.
5. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 2008; 452:230-3.
6. Mazurek S. Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells. *Int J Biochem Cell Biol* 2011; 43:969-80.
7. Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci Signal* 2009; 2:ra73.
8. Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 2008; 452:181-6.
9. Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang JK, Shen M, et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* 2011; 334:1278-83.
10. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009; 324:1029-33.
11. Hoshino A, Hirst JA, Fujii H. Regulation of cell proliferation by interleukin-3-induced nuclear translocation of pyruvate kinase. *J Biol Chem* 2007; 282:17706-11.
12. Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W, et al. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. *Nature* 2011; 480:118-22.
13. Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* 2011; 145:732-44.
14. Gao X, Wang H, Yang JJ, Liu X, Liu ZR. Pyruvate Kinase M2 Regulates Gene Transcription by Acting as a Protein Kinase. *Mol Cell* 2012.
15. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat Rev Immunol* 2007; 7:41-51.
16. Li N, Grivennikov SI, Karin M. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. *Cancer Cell* 2011; 19:429-31.
17. Demaria M, Giorgi C, Lebedzinska M, Esposito G, D'Angeli L, Bartoli A, et al. A STAT3-mediated metabolic switch is involved in tumour transformation and STAT3 addiction. *Aging (Albany NY)* 2010; 2:823-42.
18. Demaria M, Poli V. From the nucleus to the mitochondria and back: the odyssey of a multitask STAT3. *Cell Cycle* 2011; 10:3221-2.
19. Demaria M, Misale S, Giorgi C, Miano V, Camporeale A, Campisi J, et al. STAT3 can serve as a hit in the process of malignant transformation of primary cells. *Cell Death Differ* 2012.
20. Vogt M, Domoszlai T, Kleshchanok D, Lehmann S, Schmitt A, Poli V, et al. The role of the N-terminal domain in dimerization and nucleocytoplasmic shuttling of latent STAT3. *J Cell Sci* 2011; 124:900-9.



21. Yang J, Huang J, Dasgupta M, Sears N, Miyagi M, Wang B, et al. Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. *Proc Natl Acad Sci U S A* 2010; 107:21499-504.